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# Optimisation of 16S rDNA amplicon sequencing protocols for microbial community profiling of anaerobic digesters

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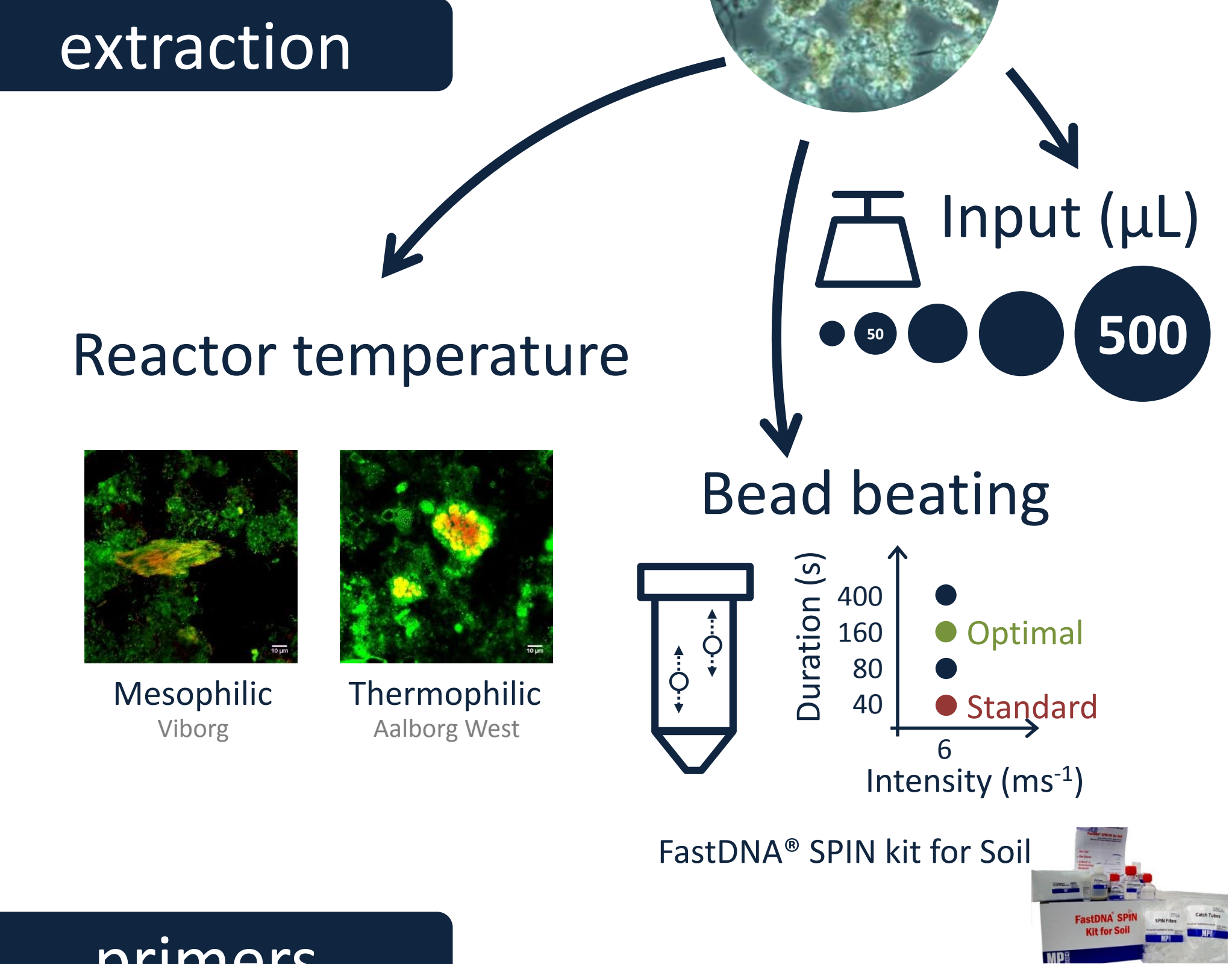
## Introduction

To understand the ecology network in anaerobic digesters it is necessary to produce a **representative** overview of the microbial community. In this study we develop a method for reliable and reproducible identification and quantification of microorganisms involved in biogas production. We test the effect of changing the parameters in a DNA extraction dependent approach to community profiling.

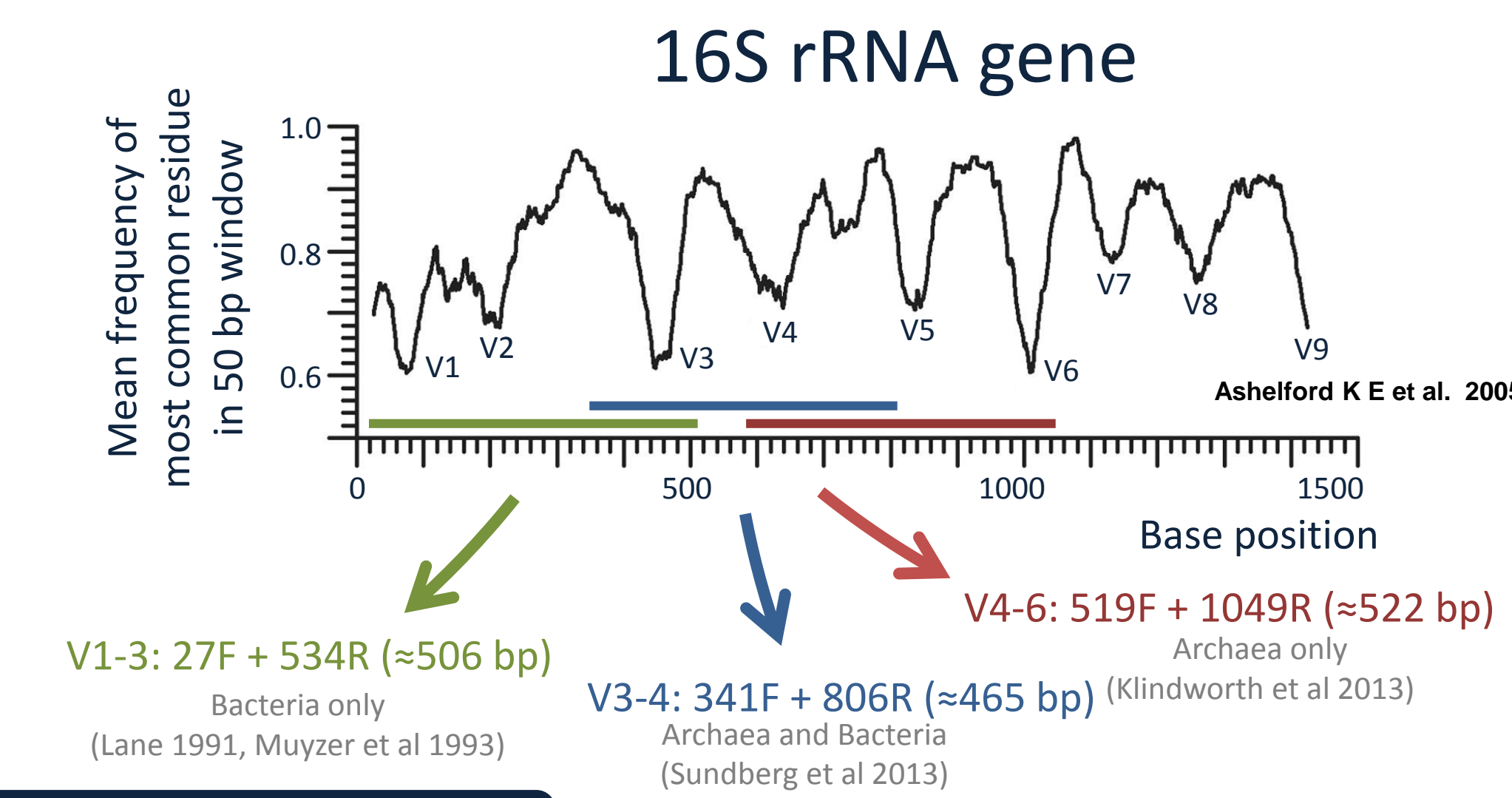
## Conclusions

- PCR independent validation is needed when conducting amplicon based studies!
- Four times the standard bead beating is recommended (160 s) in order to capture the microorganisms with relatively tough cell walls.
- The Sundberg *et al* (2013) primer set seems promising for capturing the overall community composition of both bacteria and archaea.
- Every step of the protocol introduces variance, particularly the DNA extraction. However, the workflow gives good reproducibility.

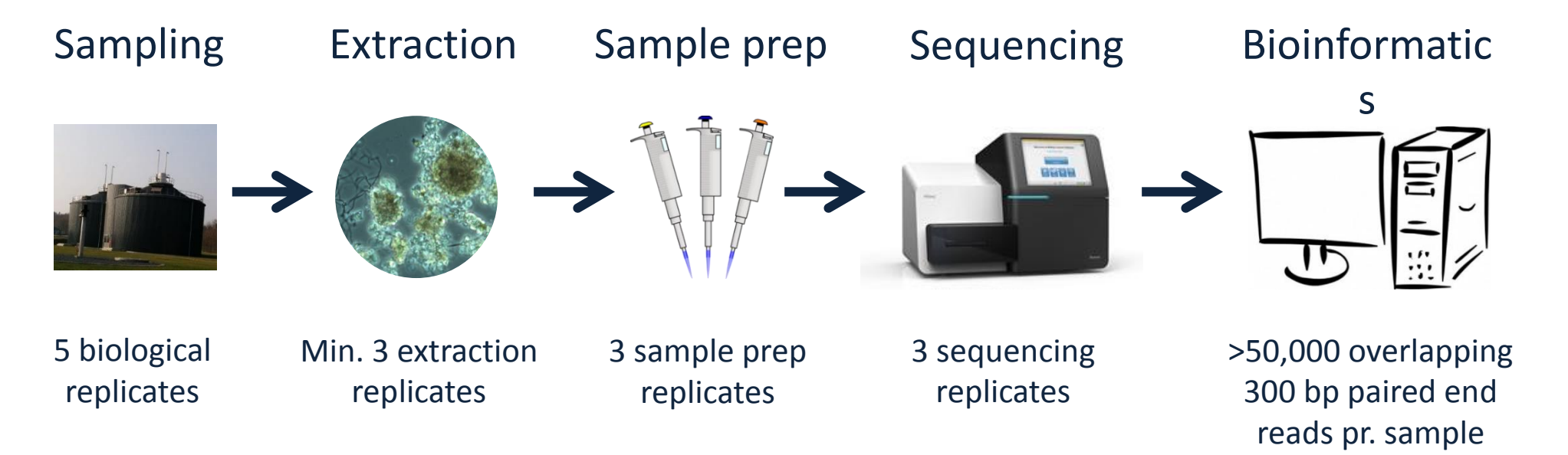
## Methods



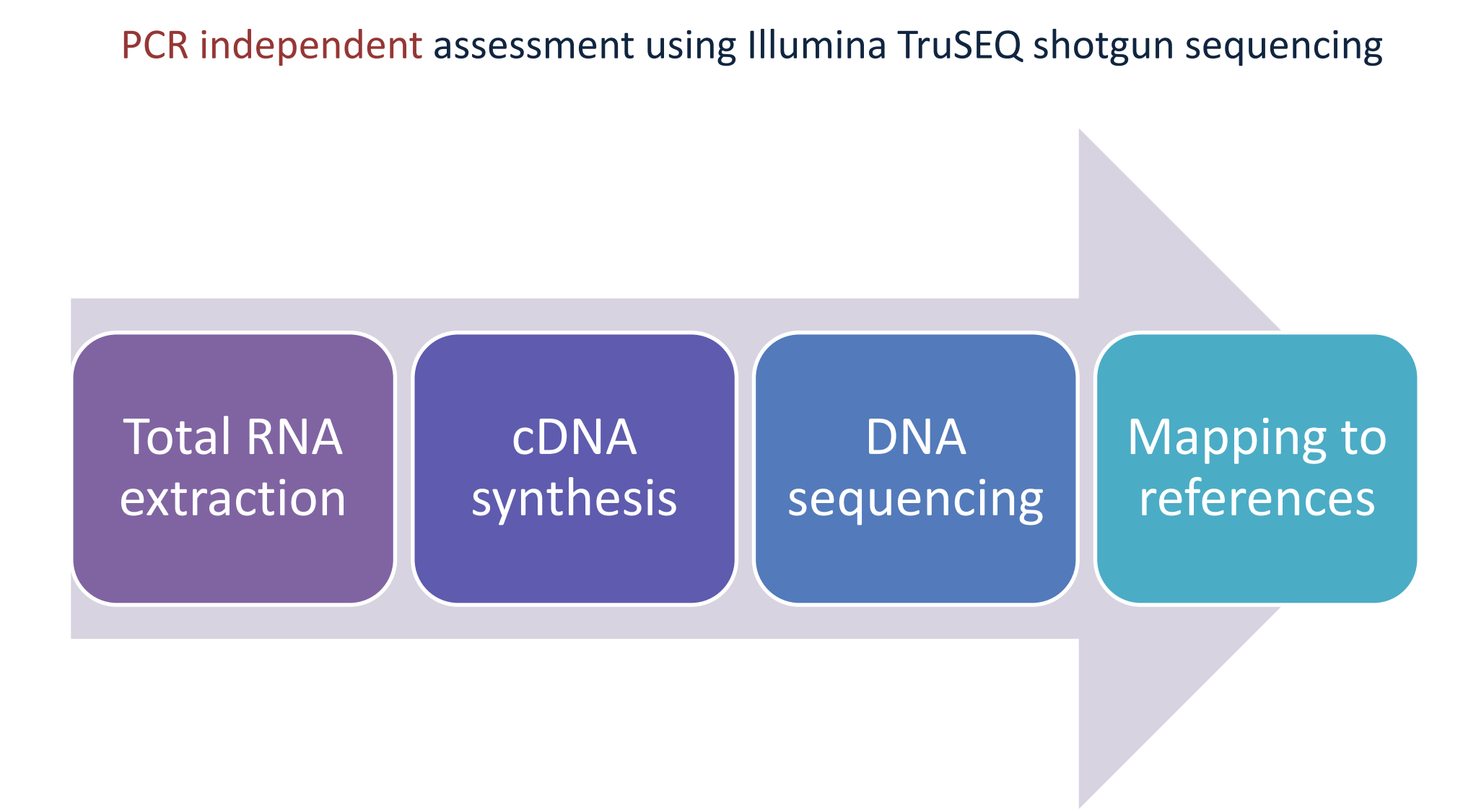
## primers



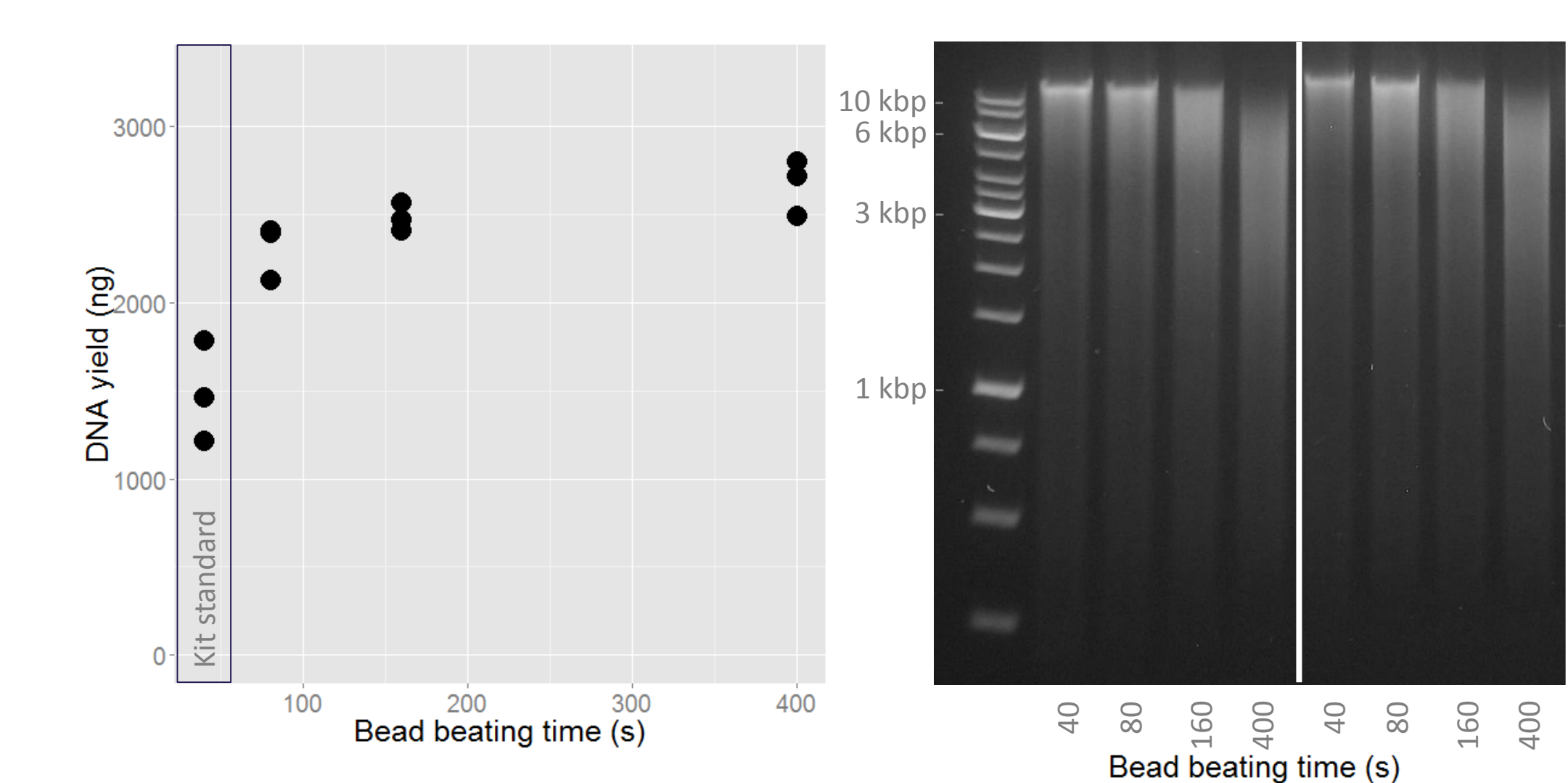
## replication



## validation



## Results



**Effect of bead beating on DNA yield.** All DNA extractions were done with 50 µL of AD sludge as input using the FastDNA® SPIN kit for Soil. The standard bead beating is 40 s.

**Effect of bead beating on DNA integrity.** At high bead beating durations the DNA is fragmented.

### Class level overview of the archaeal population

	V3-V4	V4-V6	cDNA reference
Methanomicrobia	85.9	23.2	96.7
Methanobacteria	9.5	47.6	3.0
Misc Crenarchaeotal Group	2.0	17.8	0.2
Thermoprotei	1.0	8.3	0.0
Thermoplasmata	1.5	2.7	0.2
Thaumarchaeota	0.0	0.4	0.0
MBGB	0.0	0.0	0.0

**Effect of primer set on the observed community structure.** DNA extractions were done with 160 s. All results are relative abundance (%). The V3-V4 (Sundberg et al (2013)) primer set seems to cover the archaeal diversity seen with cDNA sequencing while the archaea specific V4-V6 (Klindworth et al (2013)) primers changes the composition dramatically. Note that the cDNA sequencing and 16S amplicon results are not directly comparable, but can be used as a rough guideline.

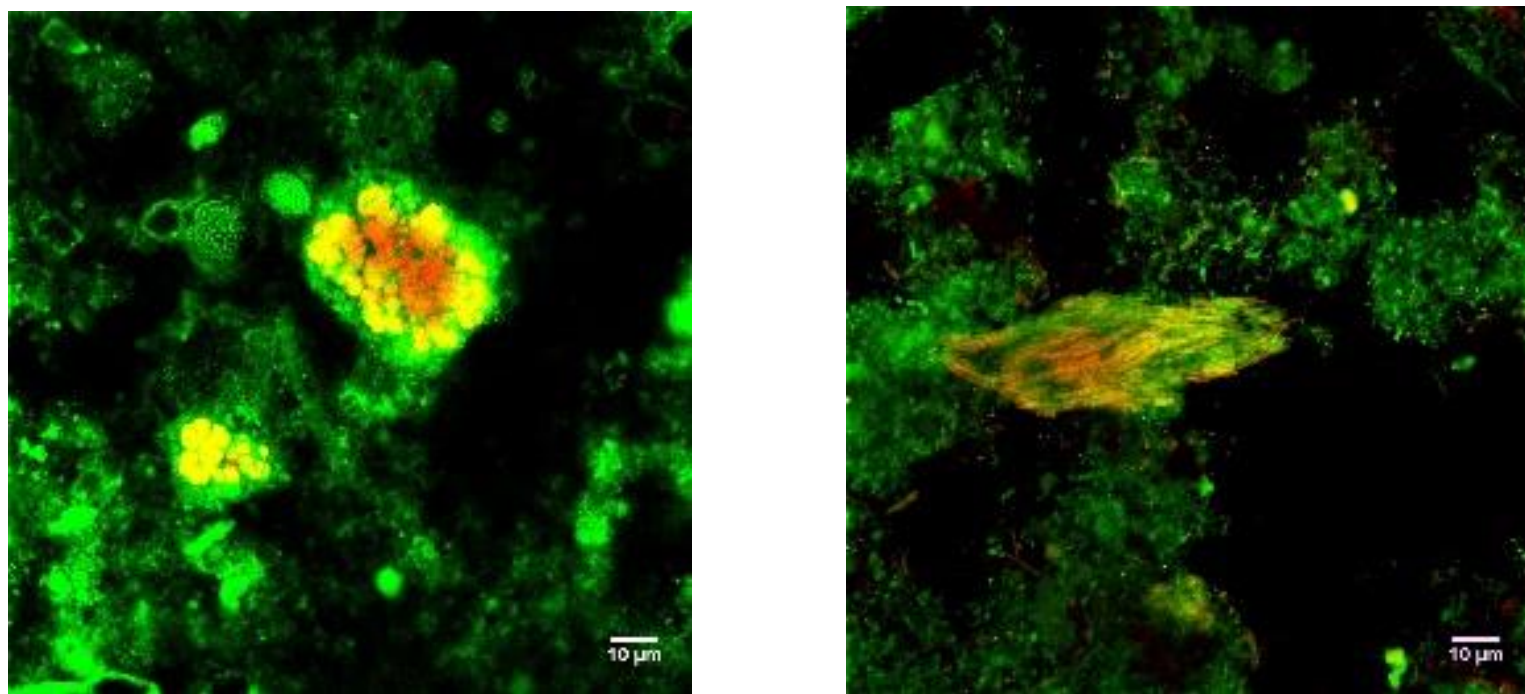
### Class level overview of the bacterial population

	V3-V4	V4-V6	cDNA reference
Chlostridia	9.1	10.7	14.6
Bacteriodes	8.7	7.3	15.1
Actinobacteria	8.8	15.6	10.2
Betaproteobacteria	15.3	9.4	7.8
Anaerolineae	5.4	6.3	10.1
Alphaproteobacteria	6.9	4.4	6.4
Deltaproteobacteria	3.0	7.2	5.0
NA	2.2	3.1	5.4
Acidimicrobia	2.7	6.3	2.9
Sphingobacteria	3.9	0.1	2.2
NA	2.2	0.0	2.5
Spirochaetes	3.8	0.9	1.0
Thermomicrobia	4.7	0.7	0.5
Gammaproteobacteria	2.0	2.3	1.1
Bacilli	0.5	3.3	1.4
Synergistia	0.8	1.5	1.6
WWE1	4.5	0.0	0.1
Brachyspirae	2.7	0.9	0.6
NA	1.5	0.0	1.5
OP8	0.6	0.5	1.9
Thermotogae	0.2	1.6	1.2
Leptospirae	0.8	2.0	0.3
Cloacamonae	0.0	3.1	0.0
Planctomycetia	0.5	1.2	0.7
Flavobacteria	1.0	0.3	0.1

**Effect of primer set on the observed community structure.** DNA extractions were done with 160 s. All results are relative abundance (%). The V3-V4 (Sundberg et al (2013)) primer set seems to cover the diversity of the bacterial domain seen with cDNA sequencing. The V1-V3 amplicon illustrates that the entire amplicon workflow has a great level of reproducibility.

### Genus level overview of the archaeal population

	V3-V4	V4-V6
f__Methanobacteriaceae	50.954	13.317
Methanolinea	0.817	38.945
Methanosaeta	1.09	32.161
Methanosarcina	26.703	0.251
Methanospirillum	0	11.558
Methanobrevibacter	0.272	1.256



**Fluorescence in situ hybridisation with archaea specific probes supports the amplicons.** *Methanosarcina* is commonly observed in high abundance in thermophilic anaerobic digesters, typically forming microcolonies, while *Methanosaeta* are often present in abundance in mesophilic reactors as short filaments. Amplicon and FISH analyses of the thermophilic and mesophilic digester samples is consistent with these observations.

